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Effects of isoprenaline and phenylephrine on energy-rich phosphate compounds and glucose-6-phosphate in smooth and cardiac muscle

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Summary

1. Tension changes produced by phenylephrine and isoprenaline have been examined in four tissues—the guinea-pig taenia coli, the longitudinal muscle strip of rabbit duodenum, the abdominal aorta of the rabbit and the rat heart.

2. Changes in the amounts of adenosine triphosphate (ATP), creatine phosphate (CP) and glucose-6-phosphate (G-6-P) associated with the addition of phenylephrine and isoprenaline have been measured in the four tissues using enzymatic fluorimetric analysis.

3. An α-adrenoceptor-mediated increase in tension (phenylephrine ; rabbit aorta) was associated with no change in the amounts of ATP, CP or G-6-P.

4. An α-adrenoceptor-mediated decrease in tension (phenylephrine ; guinea-pig taenia coli and rabbit duodenum) was associated with no change in the amounts of ATP, CP or G-6-P.

5. A β-adrenoceptor-mediated increase in force of contraction (phenylephrine and isoprenaline ; rat heart) was associated with a reduction in the amounts of ATP and CP and an increase in the amount of G-6-P.

6. A β-adrenoceptor-mediated decrease in tension (isoprenaline ; guinea-pig taenia coli and rabbit duodenum) was associated with an increase in the amounts of ATP and CP and no change in the amount of G-6-P.

7. β-Adrenoceptor-mediated metabolic changes were antagonized by propranolol.

Introduction

Bueding, Bülbring, Gercken, Hawkins & Kuriyama (1967) have shown that an increase in the amounts of adenosine triphosphate (ATP) and creatine phosphate (CP) accompanies the relaxation produced by adrenaline in guinea-pig taenia coli. The taenia contains both α- and β-adrenoceptors, both of which mediate relaxation (Brody & Diamond, 1967 ; Andersson & Mohme-Lundholm, 1968 ; Weisbrodt, Hug & Bass, 1969).

In this study, changes in the amounts of adenosine triphosphate (ATP), creatine phosphate (CP) and glucose-6-phosphate (G-6-P) have been measured in four tissues which provide examples of α-adrenoceptor-mediated excitation and inhibition and β-adrenoceptor-mediated excitation and inhibition. In order to distinguish between the effects mediated by each type of receptor, phenylephrine and isoprenaline were

used as agonists (Levy & Ahlquist, 1967). Using this approach, it was hoped to show whether any metabolic changes were dependent on the nature of the physiological response or the type of receptor involved.

A preliminary account of these observations has been given to the British Pharmacological Society (Weston, 1970).

Methods

Preparation of isolated tissues

Guinea-pig taenia coli

Guinea-pigs of either sex and weighing 250-450 g were killed by stunning and bleeding. Lengths (approximately 2 cm) of the dorsal and ventral taeniae coli were removed.

Longitudinal muscle strip of rabbit duodenum

New Zealand White rabbits of either sex and weighing 2–3 kg were killed by stunning and bleeding. A segment of duodenum was removed and incisions were made in the tissue as described by Ambache (1954). Lengths (approximately 2 cm) of the longitudinal muscle layer were then separated from the circular muscle by gentle stroking along the length of the segment with cotton wool soaked in Krebs solution.

Rabbit aortic strip

The abdominal aorta, from the renal vessels to its bifurcation in the pelvis, was removed from many of the rabbits used above. Segments of aorta were cut spirally into strips (Furchgott & Bhadrakom, 1953).

Rat heart

Hearts were removed from rats of either sex weighing 250–450 g and which had been killed by stunning and bleeding.

Measurement of rate and tension changes

Tissues from the rabbit and the guinea-pig were set up in 20 ml tissue baths containing Krebs solution maintained at 37° C and bubbled with a mixture of 95% oxygen and 5% carbon dioxide. Tension changes were recorded using a force-displacement transducer (Ether UF1) and pen recorder (Rikadenki).

Rat hearts were mounted and measurements of rate and tension made as described by Beckett (1970). Using this method of transverse recording, a signal of simple waveform was obtained from the right ventricle allowing accurate triggering of the ratemeter.

Stretching the tissues

The technique of Bülbring & Kuriyama (1963) was used to provide standard conditions within a group of tissues (with the exception of the rat heart) by stretching the tissue to a given length in relation to its weight (W:L ratio, mg/mm). The W:L ratios used were selected on the basis of preliminary experiments.

Tissue bath experiments

All tissues were blotted and weighed on a torsion balance before setting up in the tissue bath. After equilibration for 1 h in Krebs solution, they were stretched to a suitable W:L ratio by movement of the transducer to which they were attached.

In the perfused rat heart experiments, the heart was mounted under a diastolic tension of 1·5 g and the tension was adjusted to 1·5 g before each drug addition.

Biochemical experiments

Rat hearts were mounted as described for the tissue bath experiments. All other tissues were mounted on stainless steel holders which allowed the tissue to be stretched. After equilibration for 1 h in Krebs solution (maintained at 37° C and bubbled with 95% oxygen and 5% carbon dioxide), tissues were stretched to an appropriate W:L ratio.

Experimental procedures

Tissue bath experiments were used to determine the approximate ED20 and ED80 of each agonist on each tissue. Experiments with increasing concentrations of phentolamine or propranolol allowed the concentration of antagonist which reduced the response to the ED80 of agonist to that to the ED20 to be calculated. The negative logarithm of this antagonist concentration was designated the pA_R. These concentrations (ED20, ED80, pA_R) were then used in the metabolic study.

The relationship between antagonist concentration and agonist concentration ratio was analysed by the method of Arunlakshana & Schild (1959). Using this method, a graph of log (agonist concentration ratio − 1) against negative log (antagonist concentration) was constructed and its slope compared with the theoretical value for competitive antagonism of −1.

All experiments with phenylephrine on the guinea-pig taenia coli and on the rabbit duodenum were conducted in the presence of a pA_{100} of propranolol against isoprenaline, the pA_{100} being defined as the negative logarithm to the base 10 of the molar concentration of antagonist which reduced the effect of a multiple dose (100) of agonist to that of a single dose. All experiments on these tissues with isoprenaline were conducted in the presence of a pA_{100} of phentolamine against phenylephrine. Antagonists were added to the Krebs solution and a 30 min equilibration period was allowed before responses to the agonist were examined.

In the taenia, the effects of phenylephrine and isoprenaline were measured by the ability of these agonists to reduce the size of the spasm produced by acetylcholine (100 nM) (approximately an ED90) administered every 3 minutes. The tissue was exposed to phenylephrine or isoprenaline 5 s or 15 s respectively before exposure to acetylcholine (100 nM). In the rabbit duodenum, the maximum reduction in the amplitude of spontaneous mechanical activity produced by a given concentration of agonist was taken as a measure of the action of both phenylephrine and isoprenaline. Successive doses of phenylephrine and isoprenaline were administered at intervals of 4 min and 5 min respectively. In the rat heart, successive doses of phenylephrine and isoprenaline were given at 10 min intervals.

With the rabbit aorta, most dose-response experiments were conducted using the cumulative method since up to 30 min elapsed between washout of phenyl-

ephrine and return to baseline tension. Changes in the amounts of ATP, CP and G-6-P in aortic strips were measured following exposure to a single concentration of phenylephrine and, for this reason, both cumulative and sequential dose-response experiments were conducted on some tissues. In the sequential experiments, successive doses of phenylephrine were administered at 30 min intervals.

Extraction and assay of ATP, CP and G-6-P

The method of extraction for all tissues used was that described by Bueding *et al.* (1967) for guinea-pig taenia coli except that the pH was controlled by means of a pH microelectrode (Jena). The time elapsing between removal of intestinal and vascular tissues from the Krebs solution and their immersion in liquid nitrogen was about 1·5 seconds. Rat hearts were immersed in a bath of liquid nitrogen whilst still perfused with Krebs solution. Immediately after immersion, perfusion was stopped and a slice of right ventricle removed for analysis.

ATP, CP and G-6-P were assayed using the enzymatic methods of Lowry, Passonneau, Hasselberger & Schulz (1964) in conjunction with a Farrand spectrofluorimeter.

Drugs and solutions

The Krebs solution used had the following composition (mM): Na^+, 143 ; K^+, 5·93 ; Ca^{++}, 2·55 ; Mg^{++}, 1·2 ; Cl^-, 125 ; HCO_3^-, 25 ; SO_4^{--}, 1·2 ; $H_2PO_4^-$, 1·18 ; dextrose, 11·1. The pH of this solution was 7·4 while bubbling with a mixture of 95% oxygen and 5% carbon dioxide.

Drugs used were acetylcholine chloride (B.D.H.) ; (−)-isoprenaline bitartrate dihydrate (Ward Blenkinsop) ; phentolamine hydrochloride (Ciba) ; (−)-phenylephrine hydrochloride (Boots) ; (±)-propranolol hydrochloride (I.C.I.).

Hexokinase, glucose-6-phosphate dehydrogenase, creatine kinase, nicotine adenine dinucleotide phosphate, adenosine 5′ diphosphate and adenosine 5′ triphosphate were obtained from Boehringer.

Statistical methods

Student's *t* test (two-tailed) was used to measure the probability of differences between mean responses arising by chance. The *t* tests were applied according to Goldstein (1967). When the *t* value fell between two probability levels, the nearer *P* value is quoted in the results ($P\frown$). The measure of variability used is the standard error.

Results

Tissue bath experiments

Guinea-pig taenia coli

All experiments with phenylephrine or isoprenaline were conducted at W:L=0·5 (equivalent to a mean resting tension of 3·8 g) and in the presence of pA_{100} concentrations of propranolol or phentolamine respectively. In these concentrations, neither antagonist affected the tissue response to acetylcholine (100 nM).

Both phenylephrine and isoprenaline produced a concentration dependent reduc-

tion in the response to acetylcholine, the onset of action of phenylephrine being more rapid than that of isoprenaline. In sufficient concentration, both agonists completely inhibited the acetylcholine-induced spasm. Phentolamine and propranolol competitively antagonized the actions of phenylephrine ($P\simeq 0{\cdot}6$) and isoprenaline ($P\simeq 0{\cdot}4$) respectively.

Rabbit duodenum

All experiments with phenylephrine or isoprenaline were conducted at W:L=1·5 (equivalent to a mean resting tension of 1·4 g) and in the presence of pA_{100} concentrations of propranolol or phentolamine respectively. In these concentrations, neither antagonist affected the amplitude or frequency of spontaneous mechanical activity. Both phenylephrine and isoprenaline produced a concentration dependent reduction in the amplitude of spontaneous activity without affecting its frequency. The action of phenylephrine was rapid in onset and of a transient nature, whilst that of isoprenaline was slower and more prolonged. Phentolamine and propranolol competitively antagonized the actions of phenylephrine ($P\simeq 0{\cdot}5$) and isoprenaline ($P\simeq 0{\cdot}6$) respectively.

Rabbit aorta

Experiments were conducted at W:L=0·8 (equivalent to a mean resting tension of 1·5 g). Phenylephrine produced a concentration dependent increase in the tension of the aortic strips. There was no difference in response when the tissue was exposed to a given concentration of phenylephrine either as an individual dose or cumulatively ($P\geqslant 0{\cdot}4$). Phentolamine was found to be a competitive antagonist of the action of phenylephrine ($P\simeq 0{\cdot}4$).

Isoprenaline produced no change in the tension of the aortic strips.

Rat hearts

Experiments were conducted with a diastolic tension of 1·5 g. Both phenylephrine and isoprenaline produced an increase in rate and systolic tension of the heart. The maximum chronotropic and inotropic responses produced by isoprenaline were greater ($P\simeq 0{\cdot}01$) than those produced by phenylephrine. For both agonists, the ED50 for the change in rate was about 10 times greater than that for the change in systolic tension. In addition, at all dose levels, the maximum chronotropic response occurred several seconds later than the maximum inotropic response.

The inotropic and chronotropic responses to both isoprenaline and phenylephrine were competitively antagonized by propranolol ($P\geqslant 0{\cdot}6$ and $P\geqslant 0{\cdot}4$ respectively). The responses to both agonists were unaffected by phentolamine (up to 150 nM).

The data obtained from all the tissue bath experiments are summarized in Table 1.

Biochemical experiments

Guinea-pig taenia coli

Acetylcholine itself produced no change in the amounts of G-6-P but caused a reduction in the amounts of ATP and CP, changes which were temporally associated

with the rise in tension of the taenia (Table 2). These changes were unaffected by the presence of either propranolol or phentolamine (thirty experiments, $P \geqslant 0{\cdot}8$). When both isoprenaline and acetylcholine were present, the reduction in the amounts of ATP and CP was less than in the presence of acetylcholine alone. The reduction in the effect of acetylcholine was dependent on the concentration of isoprenaline (Table 3). In the presence of propranolol (100 nM), the effects of isoprenaline (40 nM) were reduced to levels not significantly different from those produced by isoprenaline (10 nM) in the absence of propranolol (fifteen experiments, $P \geqslant 0{\cdot}8$).

The interaction between acetylcholine and the two selected concentrations of phenylephrine (10 nM and 40 nM) was investigated at three times (5 s, 15 s and 25 s after exposure to acetylcholine) chosen to precede and include the peak of the resultant spasm. The presence of phenylephrine did not significantly affect the reduction in the amounts of ATP and CP produced by acetylcholine alone (fifteen experiments, $P \geqslant 0{\cdot}3$).

TABLE 1. *Summary of data obtained from tissue bath experiments and used subsequently in the metabolic study*

	Isoprenaline				Phenylephrine			
			Propranolol				Phentolamine	
	ED 20	ED 80	pA_R	pA_{100}	ED 20	ED 80	pA_R	pA_{100}
Guinea-pig taenia coli	10 nM	40 nM	7·0 (100 nM)	5·62 (2·4 μM)	10 nM	40 nM	7·15 (70 nM)	5·82 (1·5 μM)
Rabbit duodenum	10 nM	160 nM	6·19 (650 nM)	5·32 (4·75 μM)	40 nM	640 nM	6·45 (350 nM)	5·60 (2·5 μM)
Rabbit aorta	No response				40 nM	640 nM	6·85 (140 nM)	6·16 (690 nM)
Rat heart*	1 pmol	100 pmol	7·90 (13 nM)	7·93 (12 nM)	1 nmol	100 nmol	7·75† (18 nM)	7·81† (16 nM)

The terms pA_R and pA_{100} are defined in **Methods.** The values for pA_R and pA_{100} are means derived from at least nine experiments. * Data refer to inotropic responses. † Data refer to concentrations of propranolol. Phentolamine did not antagonize the actions of phenylephrine on rat heart.

TABLE 2. *Effect of acetylcholine* (100 *nM*) *on the time course of the tension response* (*% of maximum response to acetylcholine* 100 *nM*) *and on energy-rich phosphate compounds* (*μmol/g wet weight*) *in guinea-pig taenia coli*

	Time after exposure to acetylcholine 100 nM (A) or to an equal volume of vehicle (C) (s)					
	5		15		50	
	C	A	C	A	C	A
ATP	1·38 ±0·06	1·37 ±0·05	1·36 ±0·04	1·21 ±0·03	1·35 ±0·05	1·20 ±0·04
	$P \simeq 0{\cdot}9$		$P \simeq 0{\cdot}01$		$P \simeq 0{\cdot}025$	
CP	2·72 ±0·11	2·69 ±0·07	2·79 ±0·07	2·52 ±0·09	2·75 ±0·08	2·51 ±0·07
	$P \simeq 0{\cdot}8$		$P \simeq 0{\cdot}025$		$P \simeq 0{\cdot}05$	
Tension	0	12·4 ± 1·9	0	95·9 ± 5·9	0	94·8 ± 6·4
	$P < 0{\cdot}001$		$P < 0{\cdot}001$		$P < 0{\cdot}001$	

Segments were equilibrated in Krebs solution for 1 h at 37° C in an unstretched condition. They were then stretched to W:L=0·5 and 10 min later exposed to acetylcholine (100 nM) or to an equal volume of vehicle. After the times stated, tissues were frozen and prepared for assay. Each value is the mean of nine experiments ± S.E. Acetylcholine produced no significant change in the amounts of G-6-P ($P \geqslant 0{\cdot}5$).

TABLE 3. *Effect of isoprenaline on the time course of the tension response (% of maximum response to acetylcholine (100 nM) and on energy-rich phosphate compounds (μmol/g wet weight) in guinea-pig taenia coli*

Isoprenaline (nM)		Time after exposure to acetylcholine (100 nM) (s) 5 A	5 I	30 A	30 I	50 A	50 I
10	ATP	1·56 ±0·04	1·52 ±0·05	1·21 ±0·06	1·38 ±0·05	1·22 ±0·03	1·39 ±0·04
		$P \simeq 0·5$		$P \simeq 0·025$		$P \simeq 0·005$	
	CP	3·08 ±0·09	3·04 ±0·11	2·54 ±0·08	2·78 ±0·07	2·51 ±0·06	2·77 ±0·09
		$P \simeq 0·8$		$P \simeq 0·05$		$P \simeq 0·025$	
	Tension	10·9 ±1·9	0	97·3 ±5·1	79·1 ±6·2	97·9 ± 5·0	78·4 ±7·5
		$P < 0·001$		$P \simeq 0·05$		$P \simeq 0·05$	
40	ATP	1·56 ±0·04	1·54 ±0·05	1·21 ±0·06	1·41 ±0·04	1·22 ±0·03	1·63 ±0·07
		$P \simeq 0·8$		$P \simeq 0·02$		$P < 0·001$	
	CP	3·08 ±0·09	3·04 ±0·06	2·54 ±0·08	2·83 ±0·07	2·51 ±0·06	3·15 ±0·10
		$P \simeq 0·7$		$P \simeq 0·01$		$P < 0·001$	
	Tension	10·9 ±1·9	0	97·3 ±5·1	4·6 ±0·8	95·9 ±5·5	7·4 ±1·1
		$P < 0·001$		$P < 0·001$		$P < 0·001$	

Segments were equilibrated for 1 h at 37° C in Krebs+phentolamine (1·5 μM) in an unstretched condition. They were then stretched to W:L=0·5. Ten minutes later, some preparations were exposed to isoprenaline (10 nM or 40 nM) (I) followed by acetylcholine (100 nM) 15 s later. Others were exposed to acetylcholine (100 nM) alone (A). After the times stated, tissues were frozen and prepared for assay. Each value is the mean of fifteen experiments ± S.E. Isoprenaline produced no significant change in the amounts of G-6-P ($P \leq 0·5$).

TABLE 4. *Effect of isoprenaline on the time course of the tension response (% reduction of spontaneous mechanical activity) and on energy-rich phosphate compounds (μmol/g wet weight) in strips of longitudinal muscle of rabbit duodenum*

Isoprenaline (nM)		Time after exposure to isoprenaline or vehicle (s) 10 C	10 I	30 C	30 I	120 C	120 I
10	ATP	1·64 ±0·06	1·71 ±0·04	1·58 ±0·04	1·88 ±0·06	1·60 ±0·05	1·70 ±0·06
		$P \simeq 0·3$		$P < 0·001$		$P \simeq 0·2$	
	CP	3·24 ±0·11	3·29 ±0·08	3·14 ±0·09	3·44 ±0·08	3·11 ±0·07	3·30 ±0·08
		$P \simeq 0·7$		$P \simeq 0·02$		$P \simeq 0·1$	
	Tension	0	5·6 ±2·4	0	14·1 ±4·9	0	6·3 ±2·4
		$P \simeq 0·025$		$P \simeq 0·005$		$P \simeq 0·02$	
160	ATP	1·64 ±0·06	1·94 ±0·05	1·58 ±0·04	2·05 ±0·07	1·60 ±0·05	2·18 ±0·04
		$P < 0·001$		$P < 0·001$		P 0·001	
	CP	3·24 ±0·11	3·58 ±0·11	3·14 ±0·09	3·71 ±0·09	3·11 ±0·07	3·88 ±0·10
		$P \simeq 0·05$		$P < 0·001$		$P < 0·001$	
	Tension	0	27·4 ±3·2	0	64·3 ±5·0	0	81·9 ±7·1
		$P < 0·001$		$P < 0·001$		$P < 0·001$	

Strips were equilibrated for 1 h at 37° C in Krebs+phentolamine (2·5 μM) in an unstretched condition. They were then stretched to W:L=1·5. Ten minutes later, preparations were exposed to isoprenaline (10 nM or 160 nM) (I) or to an equal volume of vehicle (C). After the times stated, tissues were frozen and prepared for assay. Each value is the mean of fifteen experiments ± S.E. Isoprenaline produced no significant change in the amounts of G-6-P ($P \geq 0·6$).

Rabbit duodenum

Exposure of longitudinal muscle strips to isoprenaline was associated with a concentration dependent increase in the amounts of ATP and CP but no change in the amount of G-6-P (Table 4). In the presence of propranolol (650 nM), the effects of isoprenaline (160 nM) were reduced to levels not significantly different from those produced by isoprenaline (10 nM) in the absence of propranolol ($P \geqslant 0.7$).

The effects of exposure to phenylephrine (40 nM and 640 nM) were investigated at three times (3 s, 6 s and 9 s after exposure to phenylephrine) chosen to precede and include the maximum mechanical effect. No changes in the amounts of ATP, CP or G-6-P were detected (fifteen experiments, $P \geqslant 0.2$).

Rabbit aorta

Aortic strips were exposed to phenylephrine (40 nM and 640 nM) and assayed 60 s, 120 s and 180 s after exposure. No significant changes in the amounts of ATP, CP or G-6-P were detected (fifteen experiments, $P \geqslant 0.3$).

TABLE 5. *Effect of isoprenaline on the time course of the tension response (% of maximum change), rate response (% of maximum change) and on energy-rich phosphate compounds and glucose-6-phosphate (μmol/g wet weight) in the rat heart*

	Time after exposure to isoprenaline (1 pmol) (I) or vehicle (C) (s)				
	5	8		12	20
	I	C	I	I	I
ATP	2·21±0·08	2·24±0·07	1·84±0·06	1·99±0·08	2·20±0·07
	$P \simeq 0.8$	$P < 0.001$		$P \simeq 0.10$	$P \simeq 0.8$
CP	3·68±0·21	3·65±0·14	3·10±0·14	3·41±0·16	3·71±0·14
	$P \simeq 0.9$	$P \simeq 0.01$		$P \simeq 0.4$	$P \simeq 0.9$
G-6-P	0	0	0·31±0·05	0·18±0·02	0·09±0·02
		$P < 0.001$		$P < 0.001$	$P < 0.001$
Tension	10·1 ±3·2	0	14·9 ±5·6	12·0 ±4·0	2·4 ±0·9
	$P \simeq 0.005$	$P \simeq 0.02$		$P \simeq 0.01$	$P \simeq 0.02$
Rate	0	0	0	0	0

	Time after exposure to isoprenaline (100 pmol) (I) or vehicle (C) (s)				
	5	10		15	25
	I	C	I	I	I
ATP	1·92±0·04	2·23±0·06	1·61±0·04	1·87±0·03	2·13±0·08
	$P \simeq 0.02$	$P < 0.001$		$P \simeq 0.01$	$P \simeq 0.4$
CP	3·03±0·12	3·70±0·15	2·58±0·10	2·94±0·13	3·49±0·12
	$P \simeq 0.05$	$P < 0.001$		$P \simeq 0.02$	$P \simeq 0.5$
G-6-P	0·14±0·02	0	0·41±0·10	0·28±0·07	0·14±0·02
	$P < 0.001$	$P < 0.001$		$P < 0.001$	$P < 0.001$
Tension	41·6 ±4·8	0	77·9 ±6·4	36·2 ±4·9	3·4 ±0·4
	$P < 0.001$	$P < 0.001$		$P < 0.001$	$P < 0.001$
Rate	8·1±1·4	0	16·4 ±2·1	22·4 ±3·3	31·5 ±5·7
	$P < 0.001$	$P < 0.001$		$P < 0.001$	$P < 0.001$

Hearts were equilibrated for 30 min in PSS at a diastolic tension of 1·5 g. Some were then exposed to isoprenaline (1 pmol or 100 pmol) and others to an equal volume of vehicle. After the times stated, the hearts were frozen and a slice of right ventricle was removed for assay. Controls were only performed at the times of maximum mechanical effect (8 s and 10 s). The significance of mean changes measured at other times has been calculated using these control values. At the times of maximum mechanical effect, each value is the mean of fifteen experiments ± S.E. At all other times each value is the mean of four experiments ± S.E.

Rat heart

Isoprenaline produced a concentration dependent decrease in the concentrations of ATP and CP in the heart and an increase in the amount of G-6-P (Table 5). The maximum measured changes in energy-rich phosphate compounds and G-6-P coincided with the maximum change in systolic tension. The maximum change in rate occurred several seconds later at a time when the amounts of ATP, CP and G-6-P were returning to control levels. In the presence of propranolol (13 nM), the effects of isoprenaline (100 pmol) were changed to levels not significantly different from those produced by isoprenaline 1 pmol in the absence of propranolol (eight experiments, $P \geqslant 0{\cdot}8$).

Phenylephrine (100 pmol and 10 nmol) produced similar changes to those described for isoprenaline. Only at the higher dose level were these changes significant (eight experiments, $P \leqslant 0{\cdot}02$). In the presence of propranolol (18 nM), the effects of phenylephrine (10 nmol) were indistinguishable from controls (eight experiments, $P \geqslant 0{\cdot}3$).

Discussion

The conditions used in this study ensured that any observed response was mediated by a single type of adrenoceptor. In the intestinal tissues, this required the presence of either propranolol or phentolamine. By selecting the abdominal aorta of the rabbit (Fleisch, Maling & Brodie, 1970), it was ensured that responses from this tissue were mediated by α-adrenoceptors and this was confirmed by the lack of activity of isoprenaline. In the rat heart, no evidence of the presence of α-adrenoceptors was found. This supported the conclusion of Nickerson & Hollenberg (1967) and Wang (1967) and contrasted with the observations of Govier (1968) in guinea-pig atria.

Mechanical changes produced by isoprenaline and phenylephrine through the mediation of β-adrenoceptors were associated with significant and concentration dependent changes in the amounts of ATP and CP. These changes were reduced by the low concentrations of propranolol selected on the basis of the tissue bath experiments and there was a close temporal relationship between the metabolic and mechanical changes. It is of interest that, in the rat heart, the metabolic changes correlated well with the inotropic but not the chronotropic responses.

It is impossible to decide, without further experiments, whether these changes in the amounts of energy-rich phosphate compounds primarily reflect a change in their rate of synthesis or utilization or both, since measurements of the total concentration of a substrate reflect the algebraic sum of the processes of synthesis and utilization. In addition, measurements of either oxygen consumption (in tissues where energy supply is by oxidative mechanisms) or of substrate turnover are required. On the basis of the results obtained by other workers using such experiments, it seems likely that the β-adrenoceptor-mediated changes observed in the present study largely reflect the requirement by the muscle contractile elements for energy-rich phosphate compounds.

In the rat heart, the reduction in the amounts of ATP and CP confirms the results of previous workers (Williamson, 1966 ; Horn, Aronson, Hess & Haugaard, 1967). In conjunction with turnover and oxygen consumption measurements, the

changes probably represent an increased utilization of ATP (and reduction in total amount) following stimulation of the contractile mechanism. The associated rise in oxygen consumption is thought to reflect a compensatory increase in oxidative ATP synthesis (Williamson, 1966). Evidence that replacement of ATP is not confined to oxidative mechanisms is provided by the increased amounts of G-6-P also observed by Horn *et al.* (1967).

Guinea-pig taenia coli synthesizes ATP by oxidative mechanisms and the relaxation produced by adrenaline in this tissue is accompanied by an increase in energy-rich phosphate compounds (Bueding *et al.*, 1967) and a reduction in oxygen consumption (Bülbring & Golenhofen, 1967). These results almost certainly reflect a decreased utilization, and subsequent decrease in the synthesis, of ATP. It is reasonable to assume that the changes produced by isoprenaline in the guinea-pig taenia coli and also the rabbit duodenum in this study can be explained in a similar manner.

An acetylcholine-induced spasm in the taenia is accompanied by a rise in oxygen consumption (Bülbring, 1953). This observation, together with the reduction in energy-rich phosphate compounds produced by acetylcholine in this study, further suggests that these changes reflect the state of the contractile system and are not direct metabolic effects of the drug itself.

Mechanical changes produced by phenylephrine through the mediation of α-adrenoceptors were not associated with any significant changes in the amounts of ATP, CP and G-6-P. A possible reason for this failure was the presence of propranolol in many of the experiments. However, no changes in the amounts of ATP, CP or G-6-P were seen in rabbit aorta in the absence of propranolol, and the presence of this antagonist did not prevent acetylcholine-induced changes in energy-rich phosphate compounds in guinea-pig taenia coli.

It has been suggested above that changes in the amounts of ATP and CP associated with β-adrenoceptor-mediated responses largely reflect the drug-induced mechanical activity or quiescence of the tissue. If this is so, it is of interest that similar metabolic changes do not accompany similar mechanical effects when these are mediated by α-adrenoceptors. It is possible that responses mediated by α-adrenoceptors are associated with a mechanism which prevents the fluctuations in energy-rich phosphate compounds which are a feature of responses mediated by β-adrenoceptors.

Experiments are in progress to measure isoprenaline- and phenylephrine-induced changes in oxygen consumption in the guinea-pig taenia coli, rabbit duodenum and rabbit aorta and it is hoped that the results obtained will shed some light on this problem.

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